A genetic approach for tackling sickle cell disease

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Abstract

SCD is a serious inherited hemoglobinopathy that was responsible for the mortality of 376000 patients in 2021. The number of infants born with this genetic disorder was raised by 13.7% within the time interval from 2000 to 2021. Its fetal complications and painful VOC episodes are associated with a reduced quality of life and hospitalization and healthcare burden. Most SCD therapies are symptom-managing focused rather than curing the disease itself. This study mainly focused on the most life-threatening complications including cardiovascular complications and acute splenic sequestration, medications managing or curing them including hydroxyurea (HU) and gene therapy. HU was found to be effective in reducing cell sickling and was associated with improvements in organ functions represented in decreased acute chest syndromes and crises requiring blood transfusion. Its drawbacks were obvious in reduced sperm count and restricting erythroid cells' growth. Curing SCD gives lentiviral Gene therapy an advantage compared to HU. However, more research should be done on developing gene therapy to find out the reason and solution of malignancies reported in some cases.

I. Introduction

The disease of sickle cell anemia (SCD) was common in Africa for about 5000 years. A story of researching, discovering complications, and finding out medications, was written by great scientists, like chemist Dr. Linus Carl Pauling, Dr. Ingram, and others, to make an evolution from calling patients "ogbanjes" in Africa to the use of gene therapy techniques to treat it [1]. The disease is related to hemoglobin mutations that cause many disorders including sickle cell anemia. This disease starts with a single mutation in hemoglobin creating sickle cell hemoglobin (HbS). As a result, the shape of blood cells (from the name of the disease, sickle cell anemia) becomes crescent-like by a process of sickling the blood cells by interactions among erythrocytes, leukocytes, thrombocytes, and vascular endothelium. That leads to a blockage in the bloodstream resulting in dangerous complications and can lead to death. The complications have different levels, and some may not require hospital visits and others require intensive care units (ICU) [12]. Cardiovascular complications and acute splenic sequestration are some of those that can lead to death in both adults and children. Cardiovascular complications are clearly related to SCD by the fact that SCD can cause blockage near the heart. These complications can lead to a low supply of oxygen to the body leading to hard pain and high anemia. Because of the body's need for oxygen for its main functions, SCD affects those functions badly. Also, the spleen has an important role in the human body as it deals with dead blood cells. So, it is easily affected by closing splenic veins, by Vaso-occlusion, resulting in a complication called acute splenic sequestration (ASS). It leads to low hemoglobin levels, anemia, enlargement in the spleen size, probably splenectomy, and a mortality rate greater than 20 %. Generally, fetal hemoglobin (HbF) is known for its ability to reduce the effect of SCD [6]. That encouraged the researchers develop a medication called hydroxyurea. This medication can solve many of the cases and people who stick to it are predicted to have a longer life than others who don't

stick to it. When the level of HbF becomes in-between 20% and 33%, SCD has no effect on the patients. However, some patients don't respond to this medication positively and others have no effects at all. Another group of patients respond negatively and some of the complications deteriorate. So, scientists recommend only the minimum effective level of this therapy to avoid these negative cases [12]. Another studied medication is lentiviral Gene therapy. It depends on the gene addition method, by lentiviral vectors, to reduce the effects of HbS. Its bad side effects are not popular, making it a recommended treatment for SCD.

II. Hemoglobins

The human hemoglobin (Hb) is the factor that controls how the human body will be. Characteristics like length, eye color, and others have resulted from its function. There are many types of hemoglobin in humans. Some of them are healthy and normal and others are abnormal and can cause many disorders [1]. Mainly, the normal hemoglobins are adult hemoglobin (HbA), fetal hemoglobin (HbF), and HbA_2. Typically, they consist of two different parts, which are Heme and Globin. Heme is a combination of an iron atom and porphyrin. The term porphyrin means a heterocyclic tetrapyrrole ring system. The four rings of porphyrin are connected, cyclically, by methene bridges [21]. Globin is made up of 2 alpha chains and 2 beta chains. This is HbA ($\alpha_2\ \beta_2$). Other normal hemoglobin types are HbA_2 ($\alpha_2\delta_2$), which has 2 delta chains instead of the beta chains, and HbF ($\alpha_2\gamma_2$), which has 2 gamma chains instead of the beta chains. The alpha chain consists of 141 amino acids and the origin of the two chains is the alpha gene cluster of chromosome 16, while beta chains are comprised of 146 amino acids and their origin is the beta gen cluster in chromosome 11 [6]. Adult hemoglobin (HbA) and fetal hemoglobin (HbF) have similar genes since they exist on chromosome 11. However, HbF production decreases in an accelerated manner after birth. In the healthy conditions, adults have 96:98%, <3.5%, and <1% of HbA, HbA_2, and HbF respectively. In addition, HbA_2 is just a minor component in the blood cells in humans. So, HbA is specifically the normal one after birth [21].

Sickle cell anemia patients have a high percentage of abnormal hemoglobin called hemoglobin S (HbS). HbS is different from HbA as HbS has a valine in the 6^{th} position in the beta chains instead of amino acid. So, this mutation causes the "sickle cell disease" [6]

III. Sickle cell anemia

i. Sickle cell anemia history

Sickle cell anemia was common in Africa for about 5000 years but that was not recorded well [1]. Some scientists believe that the origin of SCD mutation happened about 70–150,000 years ago. It is known that the term "ogbanjes" was used by African people to describe weak babies who were sickle cell anemia patients in the distant past [6]. Over the last two centuries, there were observations on humans and animals that led to the full discovery of sickle cell anemia. Firstly, in 1840, at the London Zoological Garden, a scientist called Gulliver noticed strange-shaped red blood cells in the blood cells of the deer in the garden [4]. They were crescent or sickle-shaped. At the same time, the white-tailed deer in North America in the forests of Michigan were dying because of Vaso-occlusive problems. In 1905, two French scientists published a report about "half-moon corpuscles" in the blood of 5% of 243 local Algerians who were anemia patients. The scientists wrongly related these sickle erythrocytes to malaria instead of SCD. Also, in 1904, a patient called Walter Clement Noel visited the hospital of Chicago Presbyterian since he was suffering from an ulcer on his ankle [1]. His physicians were James Bryan Herrick and his intern Ernest Eddward Irons [6]. After checking Noel's blood, Dr. Irons noticed strange blood cells and named them "peculiar, elongated, and sickle-shaped". So, by the end of 1910, Dr. Herrick connected the abnormalities in the red blood cells' shapes to the sickle cell anemia disease when he reported on "Peculiar Elongated and

Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia" in the "Archives of Internal Medicine". That was the first time to do so, as a result, 1910 is described as the discovery year of sickle cell anemia disease [1]. The first scientist who denominate the disease by "sickle cell anemia" was Verne Raheem Mason in 1922 [6]. In 1927, it was discovered that removing oxygen makes red blood cells of patients, with sickle cell anemia, sickle. In addition, some of the patients' families' blood cells were sickling by removing oxygen although they had no symptoms. So, those people were called "sickle traits". That was the first discovered cause-effect relationship between the sickling of sickle cells and acidic conditions and low oxygen, by two scientists called Hahn and Gillespie [10]. In 1949, other two physicians, called Dr. James V. Neel and Col. E. A. Beet, proved that sickle cell anemia is an inherited disease that the patients are homozygous and carry two alleles of sickle cell anemia while sickle cell traits are heterozygous and carry one allele of sickle cell anemia [1]. In the same year, the famous chemist Dr. Linus Carl Pauling with his co-worker Dr. Harvey discovered the cause-effect relationship between an abnormality in the chemical structure of hemoglobin molecule and sickle cell anemia [10]. Sickle cell anemia is the first disease that took the name "molecular disease". This term was coined by Dr. Linus Carl Pauling and Dr. Harvey after their findings. Thanks to this discovery, Dr. Linus Pauling received a Nobel Prize in 1954 [1]. After two years, the details of the irregularity of hemoglobin in sickle cell patients were discovered clearly by a scientist called Dr. Ingram. In 1956, he found that the 6^{th} position of the amino acid chain has amino acid valine instead of glutamic. Counting from the amino-terminal, that 6^{th} position was the only strange one on the whole amino acid chain of SCD patients' blood [6]. That was one of the most important discoveries in sickle cell anemia. In the following years, more details were discovered, and the complications were clearly related to the disease itself. It was found that the mutation of glutamine-to-valine substitution happens in the beta-globin chain at chromosome 11. To ensure supporting SCD patients and their families Dr. Charles F. Whitten in the 1970s helped the "Sickle Cell Disease Association of America" (SCDAA) to be founded. Nowadays, 90% and 50% of SCD patients can live to the age of 20 and 50 respectively. The average age of SCD patients is about 58 years for women and 53 for men. This indicates the improvement in curing and supporting SCD patients. Because in the past, "ogbanjes" were dying at an early age [1].

ii. Pathophysiology of SCD

The pathophysiology of sickle cell anemia starts with the arising of Sickle cell hemoglobin (HbS). That happens because of the mutation in the hemoglobin (Hb) beta chain, as at the 6^{th} position, it has the amino acid valine, while the normal one is glutamate. This glutamine-to-valine substitution occurs as a result of an original mutation when the 6^{th} codon in the beta-globin chain has thymine instead of adenine [25]. This mutation creates the HbS and enables it to form polymers under deoxy conditions [13]. In addition, it causes abnormality in molecular stability and solubility. The deoxy conditions result in forming a polymer, increasing its viscosity, and decreasing solubility. Also, the deoxy conditions make HbS form tactiods, a gel-like material [1]. There is an equilibrium between the tactiods and the liquid-soluble form of HbS. This equilibrium is affected by the concentration of HbS, the existence of other hemoglobins, and oxygen tension. Oxygen tension affects the formation of the polymer. Polymer formation happens only in the deoxy state and the presence of oxygen increases the liquid state. In addition, the tactiods form of HbS happens when the concentration of HbS is greater than 20.8 g/dL. The existence of Fetal hemoglobin (HbF) can cause decreasing in the tactiods formation. So, patients that have high levels of HbF have weaker disease than patients that have low HbF levels [1]. So, HbS effects do not occur clearly until the age of six months to 2 years after declining the level of HbF. Also, people who are compound heterozygotes for HbS have one-third concentration of HbF, of all hemoglobins,

which is the needed concentration for protecting their cells from deoxy-induced damage [1]. HbS causes chronic anemia in patients as the life cycle of their blood cells is 10-20 days instead of the normal life cycle, of 90-120 days [12]. The upregulation and expression of endothelial adhesion molecules, the formation of dense red cells, and repeated sickling of the red blood cells cause one of the most dangerous processes which is Vaso-occlusion [1]. Many different reasons affect this process [18]. One of the most important reasons is the formation, or expression, of "adhesion molecules" on blood cells. Cells adhere to each other and to the vascular endothelium [10]. When this process happens in postcapillary venules, it increases the times of microvascular transit [1]. Stasis is caused by increased leukocyte recruitment and Inflammatory activation of endothelium in addition to the adhesion of sickle red cells. Many adhesion molecules are expressed on sickle cells. For example, CD36, α-4-β-1 integrin, intercellular adhesion molecule-4 (ICAM-4), and Basal cell adhesion molecule (B-CAM) are all formed on the sickle red cells [10]. During the crisis, there is a huge, expressed amount of phosphatidylserine (PS) on the sickle cells' membrane. That leads to formatting microparticles (MP) and contributes to increasing the crisis. Microparticles are used as a measurement of the activity of the disease of sickle cell anemia [18]. Vascular cell adhesion molecule-1 (VCAM-1) increases on the endothelial cells with the help of interleukin-18 (IL-18) and tumor necrosis factor alpha (TNF-α) in inflammatory processes. There is another way that leads to erythrocyte-endothelium adhesion. That happens by molecules called "bridging molecules". Von Willebrand factor (vWF), thrombospondin (TSP), and fibronectin (FN) are some of those molecules that help erythrocyte-endothelium adhesion [18]. Nitric oxide (NO) existence helps to the sickling the red blood cells which in turn leads to hemolysis. Hemolysis contributes to the existence of free hemoglobin and further leads to Vaso-occlusion. Reticulocytes are usually released by hemolysis [18]. In addition, α-4-β-1 integrin and ICAM-4 are common adhesive molecules in reticulocytes which have very large amounts of them. In fact, erythrocytes bind to reticulocytes and endothelium in sickle cell anemia because of ICAM-4's ability to bind to not only α-4-β-1 integrin, but also very late antigen (VLA-4) [1]. α-4-β-1 integrin has the ability to bind to many kinds of adhesive proteins, or molecules, such as vWF, thrombospondin (TSP), ICAM-4, and laminin in its soluble case. However, it is found that its antibodies can inhibit the adhesion of sickle erythrocytes.

All these adhesion processes lead to many crises and can damage the internal organs. The most affected organs are the lungs, the heart, and the kidneys. In babyhood, sickle cell anemia can affect them by frequent infection because of Haemophilus influenza, salmonella, and streptococcus pneumonia. Also, it can lead to a of the dorsum of the feet and hands, which is called dactylitis [17]. So, all these steps and pathophysiology from the mutations that lead to all these complications make sickle cell anemia one of the most dangerous diseases.

IV. Complications

i-insight

The slender biconcave shape of RBCs gives them the ability to gather forming rouleaux, preventing individual RBCs from clumping together in micro-blood vessels to avoid Vaso-occlusion. Furthermore, this slender shape results in a noticeable strength and elasticity obvious when they squeeze through distorted narrow blood capillaries [16]. SCD alters the RBCs' shape resulting in impaired physiology. These sickled cells are broken down prematurely by the spleen resulting in sickle cell anemia. Also, they form multi-cell adhesions combining with leukocytes and blood platelets clogging narrow blood vessels (Vaso-occlusion) giving rise to acute pain crises and depriving body organs and tissues of proper blood circulation, depriving organs of their adequate supply of nutrients and oxygen, leading to organ damage (more significantly noticed on the spleen) and eventually organ failure.

A study conducted on 8521 SCD patients (through a follow-up interval of 2.7 years) showed that Vaso – occlusive crises (VOC) are the primary reason behind SCD care utilization and medical contact like ER visits and inpatient admissions where the study recorded an average of 2.79 VOCs per patients in the first follow up year, 0.90 of which were handled in an ER setting. In addition to 0.51 VOCs handled in an outpatient setting, 0.24 VOCs were handled in an office setting, and 0.09 VOCs were handled in other settings such as a pharmacy.

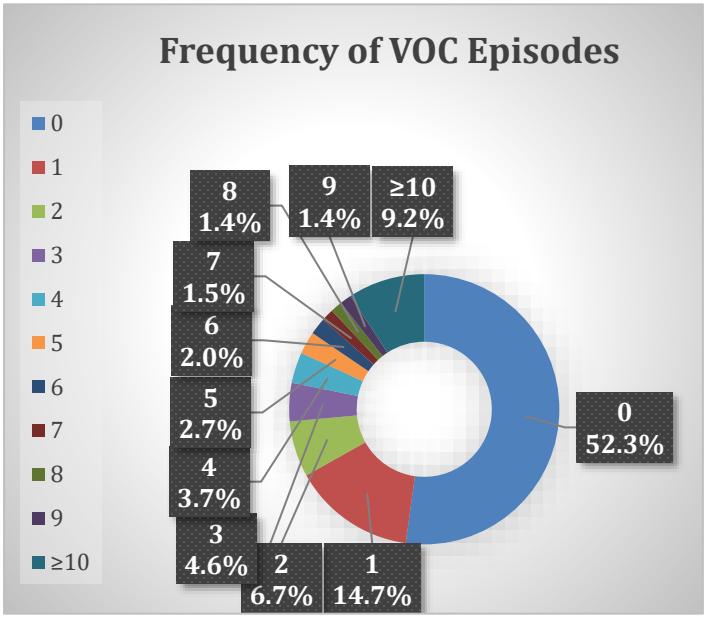

Figure 1: The frequency VOCs endured by patients during the first year of follow up.

In the first year of follow-up, 3,493 ER visits and 1,705 hospital visits related to VOC episodes and SCD complications were identified. For about 85% of those ER visits and hospitalizations, VOCs were the primary reason for admission, while the rest 15% had SCD complications as the primary reason.

Considering the four SCD genotypes, HbSS and HbSβ^0-thalassemia are known to be the most severe ones and are termed as sickle cell anemia (SCA), while HbSC and HbSβ^+ are more benign (severity variation from one case to another must be considered) [7]. Even patients with milder SCD forms typically suffer from painful crises in addition to other severe complications including avascular necrosis (AVN), acute chest syndromes (ACS), and kidney failure. Further complications include priapism, strokes, leg ulcers, asthma, chronic pulmonary hypertension, and sudden death as well (all of which are "hemolysis-endothelial dysfunction" sub-phenotype-associated complications) [20]. Current medications depend on managing the disease symptoms. However, researchers are working on a lentiviral gene therapy. SCD medication will be covered in a later section. Before that, the most important complications need to be addressed in more detail.

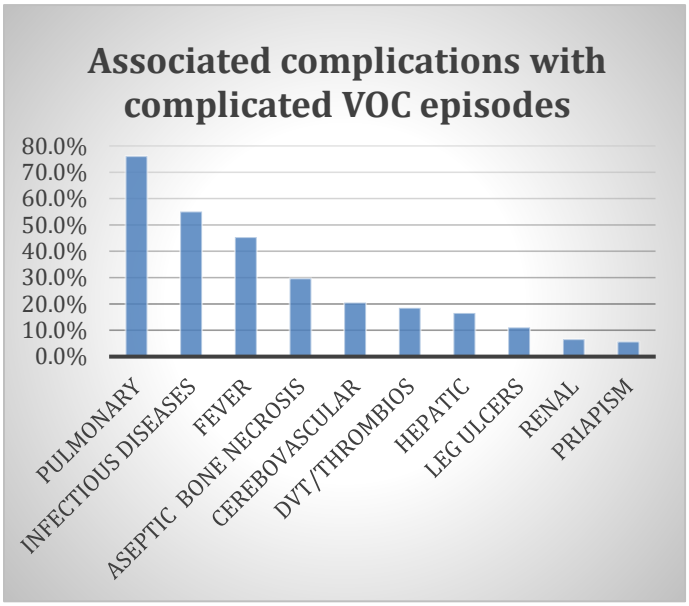

Figure 2: the top SCD complications associated with complicated VOC episodes (according to primary and secondary diagnosis claims).

ii-Cardiovascular complications

SCD is a vascular disorder, mainly driven by interactions between endothelium and the blood formed elements including sickled erythrocytes, leukocytes, inflammatory proteins, …… etc. This leads to VOC episodes and severe organ damage. Cardiovascular and endothelial changes driven by SCD differ a bit from those of non-hemolytic anemia. In patients suffering from anemia, the cardiac output (CO) increases to compensate for the low oxygen-carrying capacity of blood, and the cardiac index rises proportionally according to the severity of anemia. This higher output is sustained by higher stroke volume rather than elevating the heart

rate or the preload. The decrease in vascular resistance accounts for this increase in stroke volume. Vasodilation of resistance arterioles, exploiting previously dormant cutaneous and muscular vessels besides new vessel growth initiated by hypoxia, and reduced blood viscosity are mainly responsible for this resistance drop. Elevated stroke volume and dropped vascular resistance give rise to other physiological changes including the renin-angiotensin-aldosterone system stimulation, salt and water retention by the kidney, and a volume overload state. Consequently, chronic volume overload triggers the dilation of all cardiac chambers, developing eccentric hypertrophy as a reaction to increased wall stress [20]. In SCD, a significant drop in vascular resistance associated with an obvious rise in cardiac index (CI = CO / body surface area) are noticed compared to those of anemia, suggesting additional factors contributing to this, potentially ischemia-induced new vessel growth along with hemolysis induced inflammation. CI gives an insight into the function of the right ventricle and dropped CIs < 2.0 L/min/m2 are associated with a high risk (>10%) of death within a year [15]. Moreover, SCD patients generally suffer from cardiomegaly along with increased left ventricular end-diastolic diameter and left ventricular mass index.

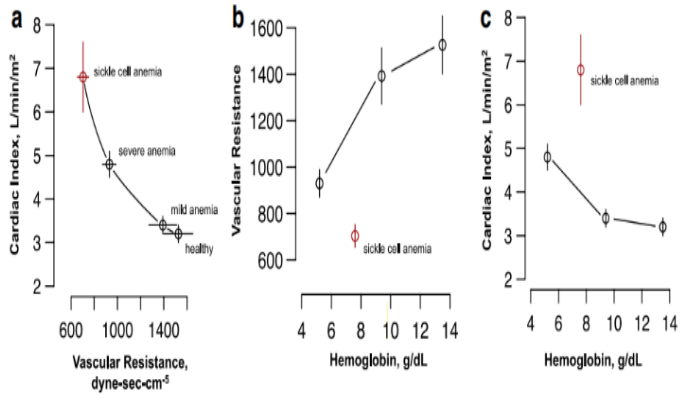

Figure 3: Comparing SCD, severe anemia, mild anemia, and healthy persons in terms of some hematological and cardiovascular indices.

SCD-associated complications are reversible and greatly depend on hemoglobin. This was obvious in Blood transfusions for men with SCD which effectively reduced the stroke volume (−10.8±4.9 mL) and the cardiac index (−0.5±0.2 L/min/m2) as well. Exactly, the reverse was seen in women, where an increase in the stroke volume was noticed (−0.5±0.2 L/min/m2). Most clinical studies of SCD patients report a normal or elevated left ventricular systolic ejection fraction. Although it seems that SCD patients don't have functional cardiomyopathy, slower shortening of circumferential myocardial muscle fibers, besides improper measures of cardiac systolic function are more common with SCD patients compared to other sorts of anemia. Additionally, impaired left ventricle relaxation and diastolic dysfunction are noticed in cases with SCD giving rise to a 3.5-fold increase in mortality. Cases suffering from SCD possess an elevated blood pressure compared to sex and age-compatible people, and higher blood pressure compared to patients with beta-thalassemia major despite having less hematocrit. Increased blood viscosity and the development of renal or vascular injury may be a satisfying explanation for that. Because of this, patients with SCD are more vulnerable to stroke and increase the probability of death.

The right shift of the Hb-oxygen dissociation curve declares a decreased hemoglobin oxygen affinity, supplying the peripheral hypoxic tissues with more oxygen and increasing oxygen extraction in anemic patients. This mechanism is mainly initiated by elevated 2,3-diphosphoglycerate levels. Sickled erythrocytes (in adults) seem to have an inefficient oxygen extraction, despite possessing plenty of (2,3-DPG) and sphingosine-1- phosphate (S-1-P) as well. Moreover, while practicing sports, oxygen extraction doesn't rise significantly compared to intact individuals, where it just increases from 24% to 39% compared to a significant rise from 25% to 50% in healthy individuals. In contrast, anemic adults were able to extract up to 80% of arterial blood oxygen content. Loss of functional capillaries, increased capillary wall thickness, less capillary transit time, and a larger count of artery-to-vein shunts may be a convincing

reason for these disorders. Oxygen extraction can be calculated from the following formula:

Oxygen extracted (mL) = arterial O2 content (mL / dL) * cardiac output (L / min) * 10dL / L * (arterialO2 content - venousO2 content) / (arterialO2 content). [6]

For some cardiac complications, there are no approved therapies till this moment. These include pulmonary hypertension (PH), whether precapillary or postcapillary disease (observed by right heart catheterization), raised pulmonary artery systolic pressure (according to Doppler echocardiography from the tricuspid regurgitant jet velocity (TRV)), left ventricular diastolic heart disease (using conventional and tissue Doppler echocardiography), raised level of N-terminal pro–B-type natriuretic peptide (NT-proBNP), dysrhythmia, and unexpected death as well. Further complications include chronic kidney disease with associated proteinuria, microalbuminuria, and hemoglobinuria.

Mainly, SCD patients developing pulmonary arterial hypertension (PAH (precapillary disease)) have progressive elevation in pulmonary vascular resistance, smooth muscle, and intimal proliferation, and in situ thrombosis as a common cause, eradicating pulmonary arterioles and resulting in a progressive heart failure in addition to reduced exercise capacity [3]. PAH is defined according to an average pulmonary artery pressure ≥ 25 mm Hg (with 15 mm Hg as the normal value), along with a left ventricular end-diastolic pressure ≤ 15 mm Hg and a pulmonary vascular resistance value ≥ 3 Wood Units. On the other hand, pulmonary venous hypertension is a result of an increase in the pressure downstream of the pulmonary arterioles and capillaries, usually associated with elevated left heart filling pressures because of diastolic or systolic heart failure. These two hemodynamic forms of PH are among the most common complications encountered by SCD patients.

RHC Results : PH vs. Non-PH : Prevalence 10.5% (5.4% precapillary)

Variable	PH (n=56)	Non-PH (n=30)	p-value
Pulm Artery Systolic Pressure	58 ± 15	31 ± 6	<0.001
Pulm Artery Diastolic Pressure	26 ± 7	13 ± 4	<0.001
Mean Pulm Artery Pressure	36 ± 9	19 ± 4	<0.001
Mixed Venous O_2 Saturation	69 ± 8	73 ± 9	0.01
TRV	3.3 ± 0.5	2.9 ± 0.4	0.001
6-minute Walk Distance	358 ± 113	441 ± 108	<0.001

Figure 4: A comparison between PH and non-PH SCD patients according to different PH indices obtained by right heart catheterization in the study of the National Institute of Health PH cohort study.

Tricuspid regurgitant jet velocity is considered a direct measurement of blood velocity returning from right ventricle to the right atrium during the systole according to Doppler echocardiography. This parameter is used for a variety of purposes including right ventricular systolic pressure calculation (equivalent to the pulmonary artery systolic pressure). It can also be used as a measure of PH. For example, a value between 2.5 m/s and 3m/s identifies 25% to 39% of patients with a mean pulmonary pressure ≥ 25 mm Hg. In addition, it is linked to Lactate dehydrogenase (LDH) and plasma-free hemoglobin, which are known to be two biomarkers of intravascular hemolysis that may lead to endothelial dysfunction and death within SCD patients [23]. Small fluctuations in the value of this parameter ≥ 2.5 are associated with an increased risk of mortality. A study published by Creteil (located in Paris) declared that TRV < 2.5 m/s is associated with less risk of death compared to TRV values ≥ 2.5 m/s which is linked to a 6.81 mortality ratio. [11]**.** Although SCD is common in sub-Saharan Africa, frequent high TRVs haven't been reported in Africa. Moreover,

higher TRVs are associated with less 6-minute walk distance [2].

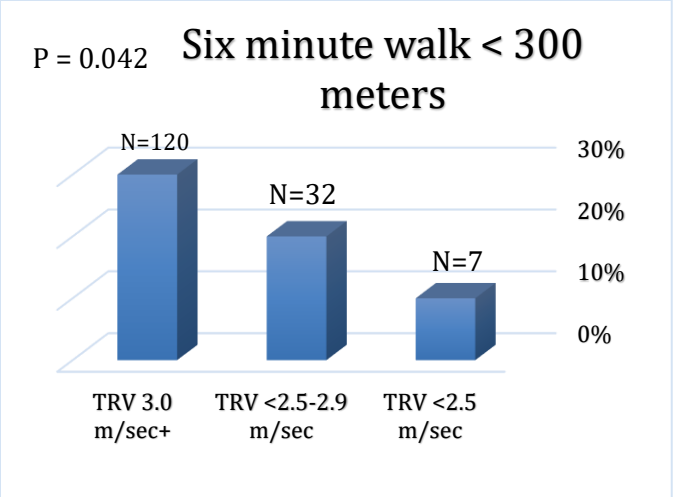

Figure 5: The percentage of patients having 6-minute walk distance within various TRV intervals.

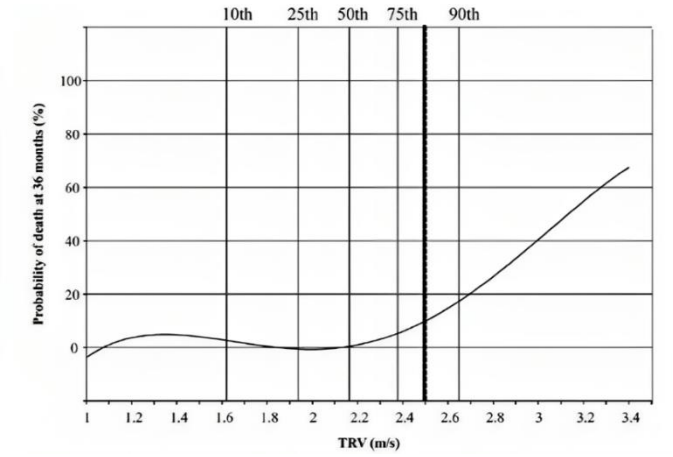

Figure 6: The relation between elevated TRVs and increased risk of mortality within a year

iii- Acute Splenic Sequestration Crisis (ASSC)

Acute Splenic Sequestration (ASS) is one of the most common complications of sickle cell anemia. Researchers define it as a drop in hemoglobin level with no less than 20%. This drop is related to an enlargement in spleen size by 2 cm at least compared with the patient's normal conditions [26]. The splenic sequestration starts with a blockage of splenic veins because of sickling blood cells in it resulting in occlusion. The blood entering the spleen is now normal and in good condition. However, the blood cells can't exit the spleen well. These events can harm the circulatory system as it leads to hypovolemia and anemia [1]. In their first decade of life, sickle cell patients face ASS and specifically between five months and 2 years. Other patients may face ASSC later in their life at about 8th decade [26]. In the crises, there are many reasons that make doctors determine if the patient has ASS or not. For example, sudden violation of anemia and splenomegaly, where the spleen becomes enlarged notability. In addition, on ASSC, the bone marrow becomes active and after blood transfusion to the patient, the size of the spleen returns to the case before ASSC [12]. There is another complication related to the spleen caused by sickle cell anemia called hypersplenism. But there is a difference between hypersplenism and Splenic Sequestration which is the fact that the spleen is always enlarged in hypersplenism, and its size doesn't regress after blood transfusions [1]. Depending on the level of riskiness, the attacks of ASS are separated into different types, which are minor attacks and major attacks. A moderate increase in spleen size and a quick decrease of the level of hemoglobin by 2–3 g/dl distinguish minor crises. While in major attacks the spleen size increases significantly, the anemia is greater than that of minor attacks as the level of hemoglobin reaches 2-3 g\dl, and it leads to hypovolemia. It is thought that the cause of ASSC is associated with upper respiratory tract infection, and specifically, infection with human Parvovirus B19 [1]. ASSC diagnosis is done clinically, and complete blood count (CBC) is usually used to indicate the level of anemia, and reticulocytosis, in addition to white blood cells (WBC) and platelets as their level decreases [12]. It is highly recommended to treat ASSC in a short time. Close monitoring and clinical evaluation should be done carefully. The intensive care unit (ICU) should keep the patients with major ASSC. Intravenous fluids should be given to the patient in ASS. Patients also should be protected by giving them H. influenzae vaccines, meningococcal, and pneumococcal. Blood transfusion is usually used with ASSC patients. However, over-transferring can

increase blood viscosity. Blood viscosity may be greater as the stuck blood in the spleen can exit it and enter circulation after blood transfusion [1]. It is important to measure the hemoglobin level after blood transfusion and keep it at its level before ASSC whatever it was [12]. After one major attack, splenectomy is popular as the spleen becomes non-functional after that. In minor attacks, splenectomy is not highly recommended [1], however, this step may be taken after two minor attacks [12]. It is common to depend on chronic blood transfusion but that has its own risks. Blood-borne infections, iron overload, hepatitis, allosensitization, and others make blood transfusion not trusted [1]. Although, on stopping blood transfusion, many patients will return to ASSC. So, the fact that splenectomy is recommended after two minor attacks is supported by the mortality rate of 20 % [12].

V.Medications

i-Hydroxyurea

Hydroxyurea (HU) is the first U.S. Food and Drug Administration FDA-approved medication for sickle cell disease. By inhibiting the ribonucleotide reductase enzyme, this cytostatic agent drains the deoxyribonucleotide reserves within the cells (used in DNA synthesis and repair). Also, this NO-releasing drug demonstrates a continuous inhibition of erythroid cell growth (reaching 20-40% within 6 days) and erythroleukemic K562 cell growth (reaching 65% within 2 days) [24]. Its hematological consequences include elevated fetal hemoglobin level (a threshold of 20% is suggested to prevent recurrent Vaso occlusions), steady state hemoglobin level, and mean cell volume (MCV) as well [27]. On the other hand, a significant drop in the populations of leukocytes, reticulocytes, blood platelets, and hemolysis markers (bilirubin and LDH for example) is noticed [19]. Comparing the morphology of erythrocytes before enduring hydroxyurea therapy, the blood film shows significantly reduced intracellular cell sickling. The main principal mechanism of action of HU is thought to be augmented fetal hemoglobin. Reduced leukocyte count caused by bone marrow suppression triggered by HU is expected to play an important role in Vasso-occlusion prevention as leukocytes are thought to be effective in VOCs initiation. The Multicenter Study of Hydroxyurea (MSH) declared a 50% reduction in the annual rate of acute pain crises along with a significant drop in the rates of acute chest syndrome and blood transfusion requiring crisis in the patients on which the study was carried out (HU was commenced at 15 mg/kg, this dose is elevated by 5 mg/kg/day to reach the maximum tolerated dose). Analogous outcomes were obtained from studies carried out on pediatric populations such as the BABY-HUG study which included children aged between 9 and 18 months. prospective non-randomized studies imported improved organ function represented in the treatment of pulmonary hypertension, secondary stroke prevention, lowered transcranial Doppler velocities, prevention of cerebral infarction, and preservation of splenic function [28]. For some of these, other studies couldn't reproduce these results. improvements including a reduction in life-threatening acute crises, less risk considering progressive organ damage, and improved life expectancy are noticed in patients who adhere to the therapy in the long term and have good clinical and hematological responses. This is supported by the studies of each of the MSH, the Brazilian pediatric cohort, and the Laikon Hospital in Athens, Greece [12]. Another study conducted on 383 patients (59 died during the follow-up period) was designed to explain whether HU-induced HbF is associated with organ damage prevention and improved survival. The patients were assigned to four quartiles depending on the maximum HbF. Only 71% of the patients in the lowest HbF quartile were alive, compared to 90% in the second quartile, 91% in the third quartile, and 86% in the highest one. The study also stated that almost all of the patients in the highest quartile (97%) were assigned to the HU group (75% of the highest quartile patients were given the recommended doses), compared to only 33% in the lowest group (and only 18% were given

the recommended doses) [9]. This is because HbF (α2γ2) lacks β-globin chains which provides it with anti- sickling effect in vitro by the interference with hemoglobin S polymerization [5].

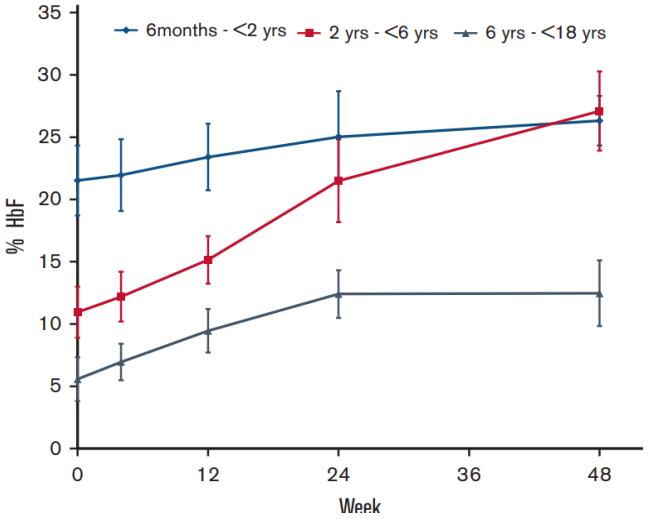

Figure 7: HU impact on HbF in patients of different ages.

Unfortunately, some SCD complications don't show improvement and may even deteriorate more during HU therapy, including childhood avascular necrosis and priapism. Moreover, 25-30% of patients suffering from SCD have suboptimal or no response at all to HU treatment [14]. Furthermore, the HUSTLE trial reported long-term toxicities in children. Also, myelosuppression is a common HU adverse effect among patients intaking higher doses and older patients. To reduce its probability of occurrence, the minimum effective dose is recommended. HU also has negative effects on spermatogenesis as it reduces sperm count and motility which normally don't return to normal even after giving off HU intake. Also, azoospermia was reported in patients treated with HU [4].

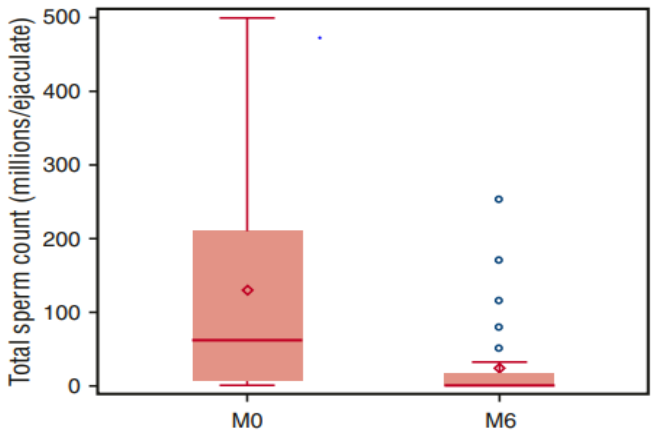

Figure 8: comparing the sperm count before and after 6 months of HU intake.

ii- Lentiviral Gene therapy

Contrary to the other SCD symptom-focused therapies, Gene therapy works to cure the disease itself. Unprecedented advances in genomic sequencing made the way for discoveries of new molecular tools for genome modification which made gene therapy a promising medication for SCD. Though bone marrow transplantation and allogenic blood are known to cure SCD, donor availability restrictions (recent studies imported that less than 25% of patients found a suitable intrafamilial donor) and graft-versus-host disease resemble significant drawbacks when they are compared to gene therapy [31]. Until now, only two techniques of gene therapy are known (gene adding and gene editing). Lentiviral gene therapy is among various gene addition therapies that use a variety of viral vector systems as lentiviral or adenoviral vectors (due to their ability to introduce new genetic information into the living cells). Recently developed methods allowed the transfer of specific genes without viral replication which increases the potential of developing a gene therapy for SCD. These gene modifications target hematopoietic stem cells (HSCs) (as they can be a life-long source of normal RBCs) and take place ex vivo in specialized facilities to avoid off-target genotoxicity that may result from systemic delivery of viral vectors. These therapies aim to minimize the effect of βS either by producing normally functioning hemoglobin or anti-sickling hemoglobin. β-globin-based gene addition strategies work on normal adult hemoglobin synthesis by incorporating

and disappears during the first year of life. BCL11A is responsible for stopping γ-globin synthesis to switch to adult hemoglobin (HbA). After this, HbF represents only < 1% of the total hemoglobin in erythrocytes. Patients with hereditary persistence of fetal hemoglobin (HPFH) along with SCD mutation have a mild SCD phenotype (due to reduced HbS concentration and HbF anti-sickling properties). Thus γ-globin-based gene addition therapy targets increasing HbF by addition of γ-globin genes. Reversing the repression of γ-globin expression is considered an alternative. In lentiviral gene therapy, HSCs are harvested from the patient, genetically modified ex vivo in cell culture, and then reinfused into the patient. To safely get through the harvesting procedure, patients are prepared through hydration and blood transfusions. Usually, patients undergo several collection cycles to harvest enough cells for gene therapy. Lentiviral vectors (which are derived from HIV) precede all vector systems in gene addition strategies [29]. However, this thought was shaken by myeloid malignancies reported from patients following the therapy [30]. The genes of the virus used to obtain a lentiviral vector have been separated into individual plasmids. The lentiviral vector is obtained from transient expression of these plasmids and is loaded only with necessary genetic c material needed to be transferred. Thus, it lacks the genes of replication and is self-inactivating due to a 3' long terminal cancellation. Because they incorporate into actively transcribed regions of the DNA, lentiviral vector offers an advantage over retroviral vector which incorporates near gene regulatory regions [32].

β-globin genes into the DNA within the cell. The drawback concerning this strategy is leaving βS mutation intact, resulting in HbS still present in erythrocytes. The addition of anti-sickling gene variants is also under development. This enables tracking the added gene expression. BA-T87Q and βAS3 are the two variants currently being discussed. Normally, γ-globin combines with α-globin to form HbF beginning in the third trimester of fetal development.

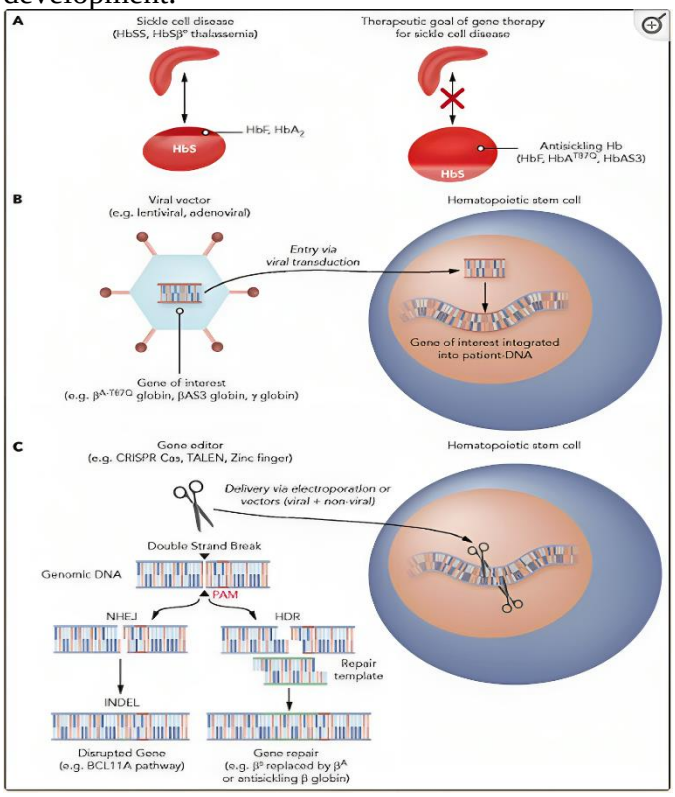

Figure 9: Gene therapy techniques.

VIII. Conclusion

HbS is the main cause of SCD. Reducing HbS and/or its effects will manage SCD riskiness. It was found that blood transfusions can lead to many side effects making it not recommended by researchers in treating SCD. Hydroxyurea is generally used to manage the complications and can't prevent all the disease. In addition, hydroxyurea can increase different disease complications while treating a specific complication. So, it was concluded that the smallest functional dose of Hu is highly recommended than overusing it. On the other hand, gene therapy techniques can treat the disease itself and can help humanity end the crisis of sickle cell anemia. In addition, it is not a chronic treatment, and patients will not need hospital visits after extracting enough hematopoietic stem cells to be gene-modified. Another key conclusion in gene therapy treatment is to use lentiviral, not retroviral, vectors to

increase the level of treatment in the therapy. So, the research question was answered by the fact that lentiviral gene therapy has a good potential to save SCD patients.

It is preferred to do future research to discover the hidden opportunities in lentiviral gene therapy to help poor patients as it is expensive nowadays. Hence, it may not be available in places like Africa where the disease spreads and the health care levels are not high to enhance such a technique. That is noticed that economic aspects were not focused on in this paper.

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